CLAIMS

1. A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a first primer included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

a second primer included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

2. The primer set according to claim 1, further comprising a third primer that hybridizes to the target nucleic acid sequence or the complementary sequence thereto,

wherein the third primer does not compete with other primers for hybridization to the target nucleic acid sequence or the complementary sequence thereto.

3. The primer set according to claim 1, wherein in the first primer, when no intervening sequence is present between the sequence (Ac') and the sequence (B'), a ratio $(X-Y)/X$ is in a range of −1.00 to 1.00, where X denotes the number of bases contained in the sequence (Ac') while Y indicates the number of bases contained in a region flanked by the sequence (A) and the sequence (B) in the target nucleic acid sequence, and when an intervening

sequence is present between the sequence (Ac') and the sequence (B') in the primer, a ratio $\{X-(Y-Y')\}/X$ is in a range of −1.00 to 1.00, where X and Y denote the same as described above, and Y' indicates the number of bases contained in the intervening sequence.

4. The primer set according to claim 1, wherein in the second primer, the folded sequence (D-Dc') has a length of 2 to 1000 nucleotides.

5. The primer set according to claim 1, wherein at least one primer included in the primer set has a solid-phase support or a site that can bind to a solid-phase support.

6. The primer set according to claim 5, wherein the solid-phase support is one selected from the group consisting of a water-insoluble organic polymer support, a water-insoluble inorganic polymer support, a synthetic polymer support, a phase transition support, a metal colloid, and a magnetic particle.

7. The primer set according to claim 5, wherein the site that can bind to a solid-phase support is selected from the group consisting of biotin, avidin, streptoavidin, an antigen, an antibody, a ligand, a receptor, a nucleic acid, and a protein.

8. A method of amplifying a target nucleic acid sequence contained in a template nucleic acid, the method comprising:

(a) preparing a template nucleic acid containing a target nucleic acid sequence;

(b) preparing a primer set according to any one of claims 1 to 7; and

(c) performing a nucleic acid amplification reaction in the presence of the template nucleic acid using the primer set.

9. The method according to claim 8, wherein the nucleic acid amplification reaction is performed isothermally.

10. The method according to claim 8, wherein a polymerase having strand displacement ability is used.

11. The method according to claim 8, wherein the nucleic acid amplification reaction is performed in the presence of a melting temperature adjusting agent.

12. The method according to claim 11, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betaine, formamide, glycerol, or a mixture of two or more of them.

13. The method according to claim 8, wherein the nucleic acid amplification reaction is performed in the presence of an enzyme stabilizing agent.

14. The method according to claim 13, wherein the enzyme stabilizing agent is trehalose, sorbitol, mannitol, or a mixture of two or more of them.

15. A method of determining the presence or absence of a mutation in a nucleic acid sequence contained in a nucleic acid sample, the method comprising:

(a) preparing a nucleic acid sample;

(b) preparing a primer set according to any one of claims 1 to 7 that is designed so that a nucleic acid sequence with or without the mutation serves as a target nucleic acid sequence, and a nucleotide residue associated with the mutation is contained in a sequence (A), a sequence (B), or a sequence (C); and

(c) performing a nucleic acid amplification reaction in the presence of the nucleic acid sample using the primer set.

16. The method according to claim 15, wherein in the process (b), a primer set is prepared that is designed so that the nucleotide residue associated with the mutation is contained in the sequence (A).

17. The method according to claim 15, wherein in the process (b), a primer set is prepared that is designed so that the nucleotide residue associated with the mutation is contained in the sequence (B).

18. The method according to claim 15, wherein in the process (b), a primer set is prepared that is designed so that the nucleotide residue associated with the mutation is contained in the sequence (C).

19. The method according to claim 15, wherein the nucleic acid amplification reaction is performed in the presence of a mismatch binding protein.

20. The method according to claim 15, wherein the nucleic acid amplification reaction is performed isothermally.

21. The method according to claim 15, wherein a polymerase having strand displacement ability is used.

22. The method according to claim 15, wherein the nucleic acid amplification reaction is performed in the presence of a melting temperature adjusting agent.

23. The method according to claim 22, wherein the melting temperature

adjusting agent is dimethyl sulfoxide, betaine, formamide, glycerol, or a mixture of two or more of them.

24. The method according to claim 15, wherein the nucleic acid amplification reaction is performed in the presence of an enzyme stabilizing agent.

25. The method according to claim 24, wherein the enzyme stabilizing agent is trehalose, sorbitol, mannitol, or a mixture of two or more of them.

26. A method of determining the presence or absence of a deletion or insertion of a sequence in a nucleic acid sequence contained in a nucleic acid sample, the method comprising:

(a) preparing a nucleic acid sample;

(b) preparing a primer set according to any one of claims 1 to 7 that is designed so that a nucleic acid sequence with or without a sequence associated with a deletion or insertion serves as a target nucleic acid sequence, and a site associated with the deletion or insertion is contained in a sequence (A), a sequence (B), or a sequence (C), or is positioned between the sequence (A) and the sequence (B) or between the sequence (A) and the sequence (C); and

(c) performing a nucleic acid amplification reaction in the presence of the nucleic acid sample using the primer set.

27. The method according to claim 26, wherein in the process (b), a primer set is prepared that is designed so that the site associated with the deletion or insertion is positioned between the sequence (A) and the sequence (B).

28. The method according to claim 26, wherein the sequence associated

with the deletion or insertion is an intronic sequence that is contained in a gene on a genome.

29. The method according to claim 26, wherein the target nucleic acid sequence is mRNA.

30. The method according to claim 26, wherein the nucleic acid amplification reaction is performed isothermally.

31. The method according to claim 26, wherein a polymerase having strand displacement ability is used.

32. The method according to claim 26, wherein the nucleic acid amplification reaction is performed in the presence of a melting temperature adjusting agent.

33. The method according to claim 32, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betaine, formamide, glycerol, or a mixture of two or more of them.

34. The method according to claim 26, wherein the nucleic acid amplification reaction is performed in the presence of an enzyme stabilizing agent.

35. The method according to claim 34, wherein the enzyme stabilizing agent is trehalose, sorbitol, mannitol, or a mixture of two or more of them.

36. A method of determining the presence or absence of a mutation in a nucleic acid sequence contained in a nucleic acid sample, the method comprising:

(a) preparing a nucleic acid sample;

(b) preparing a primer set that allows a target nucleic acid sequence containing a site associated with a mutation to be amplified, the primer set being designed so that when at least one primer included in the primer set hybridizes to a nucleic acid sequence contained in the nucleic acid sample or a complementary sequence thereto, at least one mismatch occurs between the at least one primer and the nucleic acid sequence or the complementary sequence thereto, depending on the presence or absence of the mutation; and

(c) performing a nucleic acid amplification reaction in the presence of a substance having mismatch recognition ability, using the primer set in which the nucleic acid sample serves as a template.

37. The method according to claim 36, wherein the primer set allows the target nucleic acid sequence to be amplified isothermally, and the nucleic acid amplification reaction is performed isothermally.

38. The method according to claim 36, wherein the substance having mismatch recognition ability is a mismatch binding protein.

39. The method according to claim 38, wherein the mismatch binding protein is MutS, MSH2, MSH6, or a mixture of two or more of them.

40. The method according to claim 36, wherein a first primer included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence.

41. The method according to claim 40, wherein the first primer is designed so that at least one mismatch occurs between the sequence (A) and the sequence (Ac'), depending on the presence or absence of the mutation.

42. The method according to claim 40, wherein the first primer is designed so that at least one mismatch occurs between the sequence (Bc) and the sequence (B'), depending on the presence or absence of the mutation.

43. The method according to claim 36, wherein a second primer included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

44. The method according to claim 43, wherein the second primer is designed so that at least one mismatch occurs between the sequence (C) and the sequence (Cc'), depending on the presence or absence of the mutation.

45. The method according to claim 36, wherein the primer set further comprises a third primer that hybridizes to the target nucleic acid sequence or a complementary sequence thereto and that does not compete with other primers for hybridization to the target nucleic acid sequence or the complementary sequence thereto.

46. The method according to claim 45, wherein the third primer is designed so that when the third primer hybridizes to the nucleic acid sequence contained in the nucleic acid sample or the complementary sequence thereto, at least one mismatch occurs between the third primer and the nucleic acid sequence or the complementary sequence thereto, depending

on the presence or absence of the mutation.

47. The method according to claim 36, wherein a polymerase having strand displacement ability is used.

48. The method according to claim 36, wherein the nucleic acid amplification reaction is performed in the presence of a melting temperature adjusting agent.

49. The method according to claim 48, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betaine, formamide, glycerol, or a mixture of two or more of them.

50. The method according to claim 36, wherein the nucleic acid amplification reaction is performed in the presence of an enzyme stabilizing agent.

51. The method according to claim 50, wherein the enzyme stabilizing agent is trehalose, sorbitol, mannitol, or a mixture of two or more of them.

52. A kit for determining the presence or absence of a mutation in a nucleic acid sequence contained in a nucleic acid sample, the kit comprising:

(a) a substance having mismatch recognition ability; and

(b) a primer set that allows a target nucleic acid sequence containing a site associated with a mutation to be amplified, at least one primer included in the primer set being designed so that when the at least one primer hybridizes to the nucleic acid sequence contained in the nucleic acid sample or a complementary sequence thereto, at least one mismatch occurs between the at least one primer and the nucleic acid sequence or the complementary sequence thereto, depending on the presence or absence of the mutation.

53. The kit according to claim 52, wherein the primer set allows the target nucleic acid sequence to be amplified isothermally.

54. The kit according to claim 52, wherein the substance having mismatch recognition ability is a mismatch binding protein.

55. The kit according to claim 54, wherein the mismatch binding protein is MutS, MSH2, MSH6, or a mixture of two or more of them.

56. The kit according to claim 52, wherein a first primer included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence.

57. The kit according to claim 56, wherein the first primer is designed so that at least one mismatch occurs between the sequence (A) and the sequence (Ac'), depending on the presence or absence of the mutation.

58. The kit according to claim 56, wherein the first primer is designed so that at least one mismatch occurs between the sequence (Bc) and the sequence (B'), depending on the presence or absence of the mutation.

59. The kit according to claim 52, wherein a second primer included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand,

two nucleic acid sequences that hybridize to each other.

60. The kit according to claim 59, wherein the second primer is designed so that at least one mismatch occurs between the sequence (C) and the sequence (Cc'), depending on the presence or absence of the mutation.

61. The kit according to claim 52, further comprising a third primer that hybridizes to the target nucleic acid sequence or a complementary sequence thereto,

wherein the third primer does not compete with other primers for hybridization to the target nucleic acid sequence or the complementary sequence thereto.

62. The kit according to claim 61, wherein the third primer is designed so that when the third primer hybridizes to the nucleic acid sequence contained in the nucleic acid sample or the complementary sequence thereto, at least one mismatch occurs between the third primer and the nucleic acid sequence or the complementary sequence thereto, depending on the presence or absence of the mutation.

63. The kit according to claim 52, further comprising a polymerase having strand displacement ability.

64. A method of determining the presence or absence of a mutation in a nucleic acid sequence contained in a nucleic acid sample, the method comprising:

(a) preparing a nucleic acid sample;

(b) preparing a primer set that allows a target nucleic acid sequence containing a site associated with a mutation to be amplified;

(c) preparing a nucleic acid fragment that hybridizes to the target

nucleic acid sequence and that is designed so that when the nucleic acid fragment hybridizes to a nucleic acid sequence contained in the nucleic acid sample or a complementary sequence thereto, at least one mismatch occurs between the nucleic acid fragment and the nucleic acid sequence or the complementary sequence thereto, depending on the presence or absence of the mutation; and

(d) performing a nucleic acid amplification reaction in the presence of a substance having mismatch recognition ability and the nucleic acid fragment, using the primer set in which the nucleic acid sample serves as a template.

65. The method according to claim 64, wherein the primer set allows the target nucleic acid sequence to be amplified isothermally, and the nucleic acid amplification reaction is performed isothermally.

66. The method according to claim 64, wherein the substance having mismatch recognition ability is a mismatch binding protein.

67. The method according to claim 66, wherein the mismatch binding protein is MutS, MSH2, MSH6, or a mixture of two or more of them.

68. The method according to claim 64, wherein a first primer included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence.

69. The method according to claim 64, wherein a second primer included

in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

70. The method according to claim 64, wherein the primer set further comprises a third primer that hybridizes to the target nucleic acid sequence or a complementary sequence thereto and that does not compete with other primers for hybridization to the target nucleic acid sequence or the complementary sequence thereto.

71. The method according to claim 64, wherein a polymerase having strand displacement ability is used.

72. The method according to claim 64, wherein the nucleic acid amplification reaction is performed in the presence of a melting temperature adjusting agent.

73. The method according to claim 72, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betaine, formamide, glycerol, or a mixture of two or more of them.

74. The method according to claim 64, wherein the nucleic acid amplification reaction is performed in the presence of an enzyme stabilizing agent.

75. The method according to claim 74, wherein the enzyme stabilizing agent is trehalose, sorbitol, mannitol, or a mixture of two or more of them.

76. A kit for determining the presence or absence of a mutation in a nucleic acid sequence contained in a nucleic acid sample, the kit comprising:

(a) a substance having mismatch recognition ability; and

(b) a primer set that allows a target nucleic acid sequence containing a site associated with a mutation to be amplified; and

(c) a nucleic acid fragment that hybridizes to the target nucleic acid sequence, the nucleic acid fragment being designed so that when the nucleic acid fragment hybridizes to the nucleic acid sequence contained in the nucleic acid sample or a complementary sequence thereto, at least one mismatch occurs between the nucleic acid fragment and the nucleic acid sequence or the complementary sequence thereto, depending on the presence or absence of the mutation.

77. The kit according to claim 76, wherein the primer set allows the target nucleic acid sequence to be amplified isothermally.

78. The kit according to claim 76, wherein the substance having mismatch recognition ability is a mismatch binding protein.

79. The kit according to claim 78, wherein the mismatch binding protein is MutS, MSH2, MSH6, or a mixture of two or more of them.

80. The kit according to claim 76, wherein a first primer included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence.

81. The kit according to claim 76, wherein a second primer included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

82. The kit according to claim 76, wherein the primer set further comprises a third primer that hybridizes to the target nucleic acid sequence or a complementary sequence thereto, and the third primer does not compete with other primers for hybridization to the target nucleic acid sequence or the complementary sequence thereto.

83. The kit according to claim 76, further comprising a polymerase having strand displacement ability.